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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/090,320	03/01/2002	Yanxiang Cao	3446	5376

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EXAMINER	
ZHOU, SHUBO	
ART UNIT	PAPER NUMBER
1631	

SHORTENED STATUTORY PERIOD OF RESPONSE	MAIL DATE	DELIVERY MODE
3 MONTHS	01/18/2007	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

If NO period for reply is specified above, the maximum statutory period will apply and will expire 6 MONTHS from the mailing date of this communication.

Office Action Summary	Application No.	Applicant(s)	
	10/090,320	CAO ET AL.	
	Examiner	Art Unit	
	Shubo (Joe) Zhou	1631	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *20 October 2006*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-6 and 10-29* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-6 and 10-29* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Applicants' amendment and request for reconsideration in the communication filed on 10/20/06 are acknowledged and the amendment entered.

Applicant's arguments in response to the previous Office action have been fully considered but they are not deemed to be persuasive. The following rejections and/or objections are reiterated from the previous Office action, mailed 7/20/06, and constitute the complete set presently being applied to the instant application. Rejections and/or objections not reiterated from the previous Office action are hereby withdrawn.

Claims 1-6 and 10-29 are currently pending and under consideration.

Claim Rejections-35 USC § 112

The following is a quotation of the **second** paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 1-6 and 10-29 are rejected under 35 U.S.C. 112 , second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

This rejection is newly applied.

The antecedent basis for the phrase "the RNA population" recited in claim 1, line 7, is not clear. While the phrase "an RNA sample" is recited in line 3, etc., there is no prior reference to an RNA population, which is interpreted as more than one RNA molecules.

The metes and bounds of the phrase "detecting the presence or absence of hybridization of the labeled cDNA fragments to the nucleic acid probes on the solid support" in claim 1, lines 15-16, are not clear. The claim first recites "an array comprising nucleic acid probes" in line 10-11, then recites "a first probe" and "a second probe" on the array in lines 12-13. It is not clear whether the "nucleic acid probes" hybridized to the labeled DNA fragments recited in line 16 refer to only the "first probe" and the "second probe" or to the "nucleic acid probes" comprised on the array as recited in lines 10-11, which could be probes other than the first and the second probes or probes including the first and the second probes as well as other probes.

Claim 3 recites "[t]he method of claim 1 wherein said RNA is ...." The phrase "said RNA" lacks clear antecedent basis. Claim 1 recites "an RNA sample," an "RNA population," "a first RNA isoform" and "a second RNA isoform," which are all RNAs. It is thus not clear which RNA is referred to by the phrase "said RNA" in claim 3.

Claim 10 recites "[t]he method of claim 1 wherein the solid support comprising nucleic acid probes is selected from the group consisting of a nucleic acid probe array, a membrane blot, a microwell, a bead, and a sample tube." The metes and bounds of the limitation are not clear because "the solid support" recited in line 1 is only part of an array whereas the first member of the group from which the solid support is supposed to be selected is a "nucleic acid probe array," which is interpreted as comprising a solid support and nucleic acid probes comprised thereon.

Clarification of the metes and bounds of the claims is requested.

Claim Rejections-35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are

such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 1-4, 6, and 10-29 are rejected under 35 U.S.C. 103(a) as being unpatentable over Lockhart et al. (US Patent No. 6,040,138, Date of Patent: Mar 21, 2000, filing date: Sep. 15, 1995) in view of Pharmacia Biotech (Molecular and Cell Biology Product Catalog, 1994) and Melloni et al. (The Journal of Histochemistry & Cytochemistry, Vol. 45, pages 755-763, 1997), further in view of Stahl et al. (The Journal of Histochemistry and Cytology, Vol. 41, pages 1735-1740, 1993).

The claims are drawn to a method of analyzing an RNA sample comprising converting the RNA into cDNAs with random primers and reverse transcriptase, which cDNAs are then hybridized to nucleic acid probes which can identify two different isoforms from a target gene a sample. The method comprises fragmenting the cDNAs for labeling.

Lockhart et al. teach a method of monitoring gene expression by hybridization of cDNAs derived from total RNA or mRNAs of biological samples by reverse transcription using oligo dT primers to high density oligonucleotide arrays. See columns 4, 11, 12. However, Lockhart et al. do not explicitly teach (1) using random primers for the reverse transcription, (2) degrading the RNA population during cDNA generation, (3) fragmenting the cDNA probes for hybridization.

Lockhart et al. also do not explicitly disclose providing isoform specific probes for mRNA isoform detection in a sample.

Pharmacia provides commercial kits for synthesizing cDNA from RNA for various purposes. Pharmacia provides TimeSaver cDNA Synthesis Kit comprising both Oligo dT primers and random hexamers. The instruction teaches that random primers are useful for making cDNAs that increase the representation of 5' end of an RNA, or for copying mRNAs lacking a poly(A) tail.

Melloni et al. teach fragmenting cDNA with restriction enzyme Dde-I before labeling for hybridization. Melloni et al. state that the procedure of using fragmented cDNA probes is more sensitive than other methods as evidenced by the fact that the procedure detected target mRNAs previously undetected by other means, and that the procure also detected mRNA at lower levels previously undetected by other methods. Melloni et al. reasoned that the possible explanation for these results is that because the fragmented cDNA probes "comprise a 'family' of cDNA probes, there may be more labeled nucleotide species available for hybridization to available target sequences within one mRNA molecule." See page 761, left column.

Stahl et al. provide a method for selection of oligonucleotide probes for detection of mRNA isoforms. See page 1735, Abstract and page 1736, left column. Stahl et al. states that using oligonucleotides for the detection of isoforms have clear advantages over cloned fragments such as low costs. See page 1735, right column.

One of ordinary skill in the art would have been motivated by pharmacia to modify the method of Lockhart et al. to use random primers in lieu of, or in addition to the oligo dT primers to take advantage of using random primers in reverse transcription so that the cDNA produced have a better representation of the 5' end of an RNA molecule as suggested by Pharmacia. One having ordinary skill in the art would also have been motivated by Melloni et al. to modify the method of Lockhart et al. to fragment the cDNAs before labeling to generate labeled cDNA

fragments to take advantage of its high sensitivity because the fragmented cDNA probes "comprise a 'family' of cDNA probes and the fragmentation results in more labeled nucleotide species available for hybridization to available target sequences within one mRNA molecule.

As to degrading the RNA population after the generation of cDNA by reverse transcription, while Lockhart et al. do not explicitly recite such a procedure, Lockhart et al. teach synthesizing first and second strand cDNA usiong a method by Sambrook et al., 1989 (see Lockhart et al., column 12). It would have been readily recognized by one of ordinary skill in the art that the process of obtaining such cDNA generated by such method would have inherently included a step of degrading the original mRNA. See Sambrook et al., pages 8.14-8.15 where it is explicitly taught that the mRNA is degraded by hydrolysis after the cDNA is generated.

As to having probes on the array to detect RNA isoforms of a gene, while Lockhart et al. do not explicitly including isoform specific probes on the array, they do disclose that a multiplicity of probes are provided on a high density array where each probe is complementary to a subsequence of the target nucleic acid. The multiplicity probes can include every different probe of length that is complementary to a subsequence of the target nucleic acid. The probes can range from about 10 to about 50 nucleotides in length. See column 5. It would have been obvious to one having ordinary skill in the art that the array would have been useful for isoform detection because with an array comprising such a multiplicity of probes with short sequences that are complementary to unique subsequences of a target gene, some of the multiplicity probes will hybridize to one isoform but not others because the probes are short (10-50 nucleotides long) and are complementary to only short subsequences. One of ordinary skill in the art would have been motivated by Stahl et al. to provide isoform-specific oligonucleotides on the array disclosed by Lockhart et al. in order to study the expression of different mRNA isoforms of a gene.

As to claim 2, which requires that the number of cDNA copies of a given sequence near the 3' end of an RNA is not more than twice the number of cDNA copies of a given sequence near the 5'end of the RNA molecule, it would have been obvious to a person having ordinary skill in the art at the time the invention was made that since the random primers used for priming the RNA into cDNA would be relatively uniformly distributed to an RNA molecule during reverse transcription, and as suggested by Pharmacia that the use of random primers increases the representation of the 5' end of an RNA molecule, the number of cDNA copies of a given sequence near the 3' end of the RNA would not be more than twice the number of cDNA copies of a given sequence near the 5'end of the RNA molecule, hence the hybridization signal detected with a probe to a 3' region of an RNA would not be more than twice the amount of signal detected with a probe to a 5' region of the RNA.

As to claims 3, 10, 15-20, which require the RNA sample comprises a particular type of RNA or from a particular source, Lockhart et al. teach that the RNA sample can be total RNA, or mRNA or poly(A)+ RNA. See columns 2-3, 10 and 11. Further, Lockhart et al. teach that the RNA sample can be from any organism, any biological tissues or cells, or clinical samples, or sections of tissues or frozen sections. See columns 11-12.

As to claim 14, which requires that the RNA sample is isolated from a prokaryotic cell, a person having ordinary skill in the art would have been motivated to use the method of Lockhart et al. and use random primer for the synthesis of cDNA from RNA of a prokaryotic source because Lockhart et al teach that their method can be used for RNA samples from any source (see above), and Pharmacia teaches that reverse transcription with random primer would be useful for copying mRNA lacking a poly(A) tail, which is the case for prokaryotic RNA.

As to claims 11-13, which require that the random primers used for reverse transcriptions are 6, 9, or 15 nucleotides in length, it would have been obvious to one of ordinary skill in the art that the exact length of the random primer can vary in the cDNA synthesis because different

length of random primers have been used in the prior art. For example, the kits of Gibco BRL and Pharmacia comprise random hexamers (6mer); Malfroy-Camine et al. (US 5,780,025, date of patent: Jul. 14, 1998) teach using random octamers in the synthesis of cDNA from RNA (see column 17); and Lader et al. (US 6,057,134) disclose using random decamers for reverse transcription to synthesize cDNA (see column 6). Thus, one of ordinary skill in the art would be motivated to try various lengths of random primers such as, 6mers, 9mers or 15mers to see whether better synthesis would be achieved.

This rejection is reiterated from the previous Office action mailed 7/20/06. Applicant's arguments filed 10/20/06 have been fully considered but they are not persuasive. The argument is on the ground that the recited references do not alone or in combination teach all the elements of the claims, such as claim 1. Specifically, applicant argues that the reference by Melloni et al. "provides no teaching or suggestion of fragmentation of cDNA." This is not found persuasive because Melloni et al. explicitly teach fragmentation of cDNA for preparation of cDNA probes throughout. For example, they state on page 757, left column:

> *The cDNA fragments were generated with the restriction endonuclease Dde I ... Dde I has a four nucleotide base recognition sequence (C/TNAG), and therefore typically recognizes and digests at a high frequency along any given length of DNA.*

Clearly Melloni et al. teach fragmentation of cDNA using frequent cutter restriction enzyme Dde I to generate smaller cDNA fragments for probe preparation.

Claim 5 is rejected under 35 U.S.C. 103(a) as being unpatentable over Lockhart et al. in view of Pharmacia Biotech, Melloni et al. and Stahl et al., as applied to claims 1-4, 6, and 10-29 above, further in view of Gibco BRL (Terminal Deoxynucleotidyl Transferase, Gibco BRL Catalog and Reference Guide, 1992).

The claim is drawn to a method of analyzing an RNA sample comprising converting the RNA into cDNAs with random primers and reverse transcriptase, which cDNAs are then fragmented and labeled by the addition of at least one labeled nucleotide using terminal transferase before being hybridized to nucleic acid probes on a solid support.

Applied to claims 1-4, 6, and 10-29 above, Lockhart et al., Pharmacia Biotech teach or suggest a method of monitoring gene expression by hybridization of cDNAs derived from total RNA or mRNAs of biological samples by reverse transcription using random primers to high density oligonucleotide arrays. However, the references do not explicitly teach that the cDNA fragments are labeled by the addition of at least one labeled nucleotide using terminal transferase.

Lockhart et al. teach that the labels of the cDNAs can be made with any of the means known to those of skill in the art such as end labeling.

Gibco BRL discloses and provides a terminal deoxynucleotidyl transferase. The instruction for the product states that the enzyme is "suitable for adding momopolymer tails to the 3' end of DNA" or "for labeling the 3' ends". See page 290.

It would have been obvious to one having ordinary skill in the art at the time the invention was made to modify the method of Lockhart et al. to use terminal transferase to end label the cDNA fragments because Lockhart et al. clearly motivates and suggests end labeling and Gibco BRL provides the terminal transferase enzyme for exactly this purpose.

This rejection is also reiterated from the previous Office action mailed 7/20/06. Applicant's arguments filed 10/20/06 have been fully considered but they are not persuasive. The argument is on the same ground as that provided above for the rejection of claims 1-4, 6, and 10-29 under 35 U.S.C. 103(a) over Lockhart et al., Pharmacia Biotech, Melloni et al. and Stahl et al.

The argument is not found persuasive for the same reasons as those set forth above in the respective section.

Conclusion

No claim is allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Shubo (Joe) Zhou, whose telephone number is 571-272-0724. The examiner can normally be reached Monday-Friday from 8 A.M. to 4 P.M. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Andrew Wang, can be reached on 571-272-0811. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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PATENT EXAMINER